AD_____

DRUG EVALUATION IN THE PLASMODIUM FALCIPARUM-AOTUS MODEL

ANNUAL REPORT

Richard N. Rossan

(For the period May 15, 1987 to May 14, 1988)

June 1, 1988

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick
Frederick, Maryland 21701-5012

Contract No. DAMD17-87-C-7163

Gorgas Memorial Laboratory
Panama, Republic of Panama

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Gorgas Memorial Laboratory; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Panama, Republic of Panama	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S.Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Contract No. DAMD17-87-C-7163
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 62770A; PROJECT NO. 3 M1 62770A870; TASK NO. AJ; WORK UNIT ACCESSION NO. 003

11. TITLE (Include Security Classification): Drug Evaluation in the Plasmodium falciparum - Aotus Model

12. PERSONAL AUTHOR(S): Richard N. Rossan

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 5/15/87 TO 5/14/88	1988 June 1	68

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	13	
06	15	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number): Plasmodium falciparum Aotus trivirgatus folic acid inhibitors RA1 reversal of chloroquine resistance calcium channel blockers

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Infections of the pyrimethamine resistant Vietnam Smith strain of Plasmodium falciparum in Aotus trivirgatus were used to evaluate the activity of four folic acid inhibitors. As expected, there was cross resistance to pyrimethamine (WR 002978), proguanil (WR 003019) and cycloguanil (WR 005473). A proguanil analog (WR 250417), however, had significant antimalarial activity. A 3-day dosage of 1.0 mg/kg cured 3 of 7 infections, 10.0 mg/kg cured 6 of 8 infections, and 50.0 mg/kg cured 4 of 4 infections.

Trials were initiated to reverse, in vivo, chloroquine resistance in infections with the Vietnam Smith and Smith/RE strains (chloroquine resistant) by the co-administration of calcium channel blockers plus chloroquine. These trials were based upon the reports of in vitro reversal of chloroquine resistance. Six calcium channel blockers, or similar acting drugs were tested. In 26 primary treatments with a channel blocker plus chloroquine, parasitemia

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mary Frances Bostian	22b. TELEPHONE (Include Area Code): 301-663-7325; 22c. OFFICE SYMBOL: SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

19. ABSTRACT (Cont'd)
were cleared in two monkeys each treated with WR 255694, verapamil, (20.0 mg/kg x 3 days) plus chloroquine (20.0 mg/kg x 3 days). The infection was cured in one Aotus. In a total of 28 repeat treatments six infections were cured. Nifedipine, WR 255695, cured four infections as follows: two each with 40.0 mg/kg (x 3 days) plus chloroquine (20.0 mg/kg x 3 days), and one with 10.0 mg/kg (x 3 days) plus chloroquine (40.0, 20.0, 20.0 mg/kg). WR 256287 cured two infections, one with 5.0 mg/kg (x 3 days) and one with 50.0 mg/kg (x 3 days); chloroquine was co-administered at 20.0 mg/kg (x 3 days).

The duration of the primary patent period and the time for parasite clearance in the animals cured of infection were equal to these parameters in untreated Aotus exhibiting self-cure. Consequently, it is difficult to assign cure to either drug action or acquired immunity, and probably resulted from a combination of both.

SUMMARY

The objective of the contract program is to evaluate experimental antimalarial drugs against Plasmodium falciparum infections experimentally induced in the Panamanian owl monkey (Aotus). For the studies in this report, the Vietnam Smith strain was used.

Four folic acid inhibitors were tested against infections of the pyrimethamine-resistant Smith strain. As anticipated, there was cross-resistance to pyrimethamine (WR 002978), proguanil (WR 00 3019), and cycloguanil (WR 005473). These drugs had no effect upon parasitemias. In contrast, a proguanil analog (WR 250417), showed significant antimalarial activity. A 3-day dosage of 1.0 mg per kg cured three infections of a total of seven treatments, 10.0 mg per kg cured six infections out of a total 8 treatments, and 50.0 mg per kg cured four infections of a total of four treatments. The antimalarial activity of this analog against infections of pyrimethamine-resistant strains of P. falciparum is significant in comparison with other folic acid inhibitors.

The majority of experiments were concerned with in vivo attempts to reverse chloroquine-resistance by the concomitant administration of a calcium channel blocker plus chloroquine. These trials were predicated upon the successful in vitro results of chloroquine-resistance reversal. It is suggested, to explain this phenomenon, that the channel blocker prevents the active efflux of chloroquine by the parasite from the erythrocyte allowing chloroquine to achieve a parasitocidal level.

Six calcium channel blockers, or similar acting drugs, were tested with chloroquine for their capacity to reverse chloroquine-resistance in infections with the Vietnam Smith or Smith/RE strain of P. falciparum. The desideratum of a trial would be to administer the two drugs in the ascending phase of the primary attack, resulting in parasite clearance and infection cure, as indicated by the absence of recrudescence. Verapamil (25.0 mg per kg x 3 days) plus chloroquine (20.0 mg per kg x 3 days) cleared the parasitemia during the primary attach in each of two Aotus. The infection in one monkey was cured; the second animal died of an intercurrent infection on day 29 post treatment. Primary treatment with other calcium channel blockers plus chloroquine was either not effective or suppressed the parasitemia.

In a total 28 repeat treatments, x infections were cured, four with nifedipine (WR 255695) plus chloroquine, and two with WR 256287 plus chloroquine. The duration of the primary patent period in the monkeys cured of their infection was equal to that in untreated monkeys that exhibit self-cure after the primary attack. It is, therefore, difficult to separate drug activity from acquired immunity as effecting cure.

The failure to achieve the desired goal of reversing chloroquine resistance in vivo as indicated by a shortening of the duration of the primary patent period, parasite clearance and infection cure may

be, in part, attributable to:

1. Inability to maintain high drug levels compatible with host viability, and/or

2. Drug metabolism in the host yielding inactive components.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Laboratory Animal Resources Commission of Life Sciences, National Research Council (NIH Publication No. 86-23, Revised 1985)

TABLE OF CONTENTS

EXPERIMENTAL PROCEDURES

The monkey-adapted Plasmodium falciparum strain, Vietnam Smith (resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine), and Vietnam Smith/RE line were used to induce experimental malaria infections in Aotus trivirgatus for the evaluation of the antimalarial efficacy of candidate drugs. Infected blood, with sodium citrate (2.5%) as the anticoagulant, from untreated Aotus was diluted appropriately with chilled saline (0.85%), such that each milliliter contained 5,000,000 parasites, and this amount was injected into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first post-inoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; < 10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Earle-Perez method and reported as the number of parasites per cmm.

Blood films from untreated Aotus, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If a recrudescence occurred, blood films were obtained again on a daily basis.

The schema depicted in Figure 1 represents the design of a typical drug evaluation study. Parasitemias were evaluated daily during the treatment period and until blood films were negative for at least seven consecutive days. The frequency of smearing was then reduced to two times per week (Monday and Thursdays or Tuesdays and Fridays). If no recrudescences occurred during a 100 day examination period, the infection was considered to have been cured.

Drug doses were calculated as mg base per kg of body weight. Stock solution of water soluble compounds, at appropriate concentrations, were prepared with distilled water and stored at 8°C for the treatment period. If a compound was water insoluble, a suspension of the requisite amount of drug was prepared daily with 0.3% methylcellulose (in distilled water).

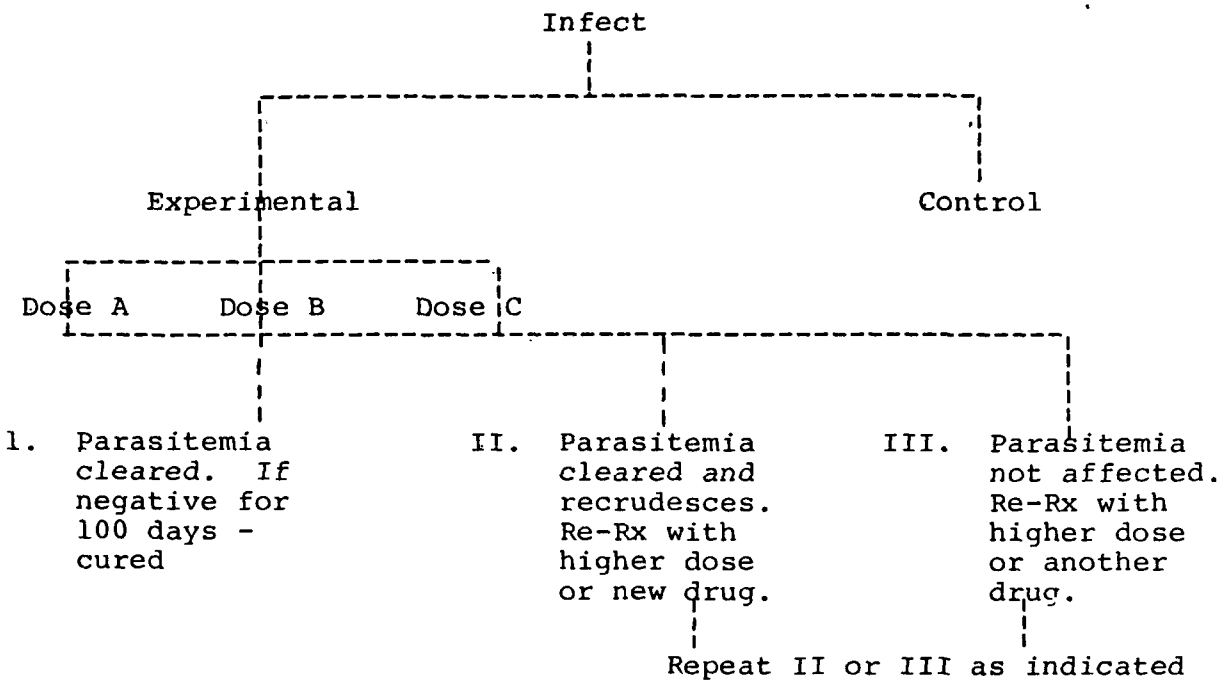
Oral administration of drugs was effected by gastric intubation with a 14 French catheter. The total amount of fluid administered, drug solution or suspension, and rinse was 14 ml.

FIGURE 1

SCHEMA FOR DRUG EVALUATION AGAINST

PLASMODIUM FALCIPARUM

INDUCED INFECTIONS IN AOTUS TRIVIRGATUS



ASSESSMENT OF THE ANTIMALARIAL ACTIVITY OF FOUR FOLIC ACID INHIBITORS AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

The emergence of resistance to the dihydrofolate reductase inhibitors, in both P. falciparum and P. vivax, has been of considerable import to the treatment and control of such infections during the past 40 years. The cross resistance among this class of drugs offers a challenge to develop a new and effective agent. Data presented in this section are concerned with the evaluation of three standard folic acid inhibitors, and a newly developed analog of proguanil, against infections of the pyrimethamine resistant Vietnam Smith strain of P. falciparum.

A. WR 005473AM (BN:BK 40388)

The data in Tables 1, 2, and 3 indicate that, at the doses used, cycloguanil was essentially inactive against Vietnam Smith infections. Suppression of parasitemia, however, did occur in four monkeys, one treated with 1.0 mg base per kg (x 3), one that received 10.0 mg base per kg (x 3), and in each of two that received 50.0 mg base per kg (x 3). The infections were re-treated with WR 250417AA, as discussed in Part D.

B. WR 003019AM (BN:BL 18309)

Proguanil, administered at doses of 1.0, 10.0, and 50.0 mg base per kg (x 3), had no effect upon parasitemias (Tables 4,5, and 6). The infections were re-treated with WR 250417AA, as shown in Part D.

C. WR 002978AK (BN:BK 39401)

In the one monkey, 12242, used as a pyrimethamine-treated control in these studies, a dose of 2.5 mg base per kg (x 3), had no effect upon parasitemia. Retreatment was initiated with the same dose, but the monkey died of malaria on the third day of treatment.

D. WR 250417AA (BN:BK 47734)

As shown in Tables 7,8, and 9, this proguanil analog was evaluated against primary parasitemias as well as treatment failures with cycloguanil and proguanil. A dose of 0.1 mg base per kg (x 3) was not effective against parasitemias Parasite clearance was uniformly effective if the three day treatment was completed.

A dose of 1.0 mg base per kg (x 3) cured 3 of 7 (43%) of the infections, 10.0 mg base per kg (x 3) cured 6 of 8 (75%) of the infections, and a dose of 50.0 mg base per kg (x 3) cured 4 of 4 (100%) of the infections.

There was no apparent toxicity of the proguanil analog at any of the doses administered.

CONCLUSION

As to be expected, there was cross resitance of this pyrimethamine resitant strain to cycloguanil and proguanil. The proguanil analog, however, was highly effective in clearing parasitemias and curing infections, either primary or retreatment. These results offer the potential of eventually using this analog against human infections resistant to dihydrofolate reductase inhibitors.

TABLE 1

DETAILED ACTIVITY OF WR 005473AM (BK 40388), CYCLOGUANIL, AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dosage mg/Kg	Day Pre-Rx	Parasitemia per cmm x 10^3									
			Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12039	1.0	11	10	114	517	429	641	296	Re-Rx, new drug			
12165	1.0	2	51	40	55	36	65	50	Re-Rx, new drug			
12138	10.0	2	80	13	8	11	51	28	Re-Rx, new drug			
12168	10.0	13	90	56	450	105	566	593	Re-Rx, new drug			
12174	50.0	1	35	24	28	22	73	57	Re-Rx, new drug			
12175	50.0	0.7	18	7	3	0.4	<0.01	<0.01	Re-Rx, new drug			
12242	2.5a)	10	102	40	517	429	551	641	Re-Rx, higher dose			
12242r	2.5a)	641	395	120	Died - malaria							

a) Pyrimethamine

TABLE 2

SUMMARY OF THE ACTIVITY OF WR 005473AM (BK 40388), CYCLOGUANIL, AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12039	1.0	+			n.a.	n.a.	Re-Rx, new drug
12165	1.0		+		n.a.	n.a.	Re-Rx, new drug
12138	10.0				n.a.	n.a.	
12168	10.0	+	+		n.a.	n.a.	Re-Rx, new drug
12174	50.0				n.a.	n.a.	
12175	50.0		+		n.a.	n.a.	Re-Rx, new drug
			+		n.a.	n.a.	Re-Rx, new drug
12242	2.5a	+			n.a.	n.a.	
12242r	2.5a	+			n.a.	n.a.	Re-Rx, new drug
					n.a.	n.a.	Re-Rx, new drug
					n.a.	n.a.	Re-Rx, same dose
							Died, malaria, Day 3 of Rx

a. Pyrimethamine

TABLE 3

ACTIVITY OF WR 005473AM (BK 40388), CYCLOGUANIL, AGAINST PLASMODIUM FALCIPARUM INFECTIONS

MALARIA	DOSE mg/kg		PRIMARY TREATMENTS		REPEAT TREATMENTS		TOTAL TREATMENTS	
STRAIN	TOTAL	DAILY	CLEARED	CURED	CLEARED	CURED	CLEARED	CURED
Smith	3.0	1.0	0/2	0/2			0/2	0/2
	30.0	10.0	0/2	0/2			0/2	0/2
	150.0	50.0	0/2	0/2			0/2	0/2

TABLE 4

DETAILED ACTIVITY OF WR 003019AM (BL 18309), PROGUANIL, AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12197	1.0	3	103	60	161	296	234	517	Re-Rx, new drug			
12326	1.0	2	45	27	142	80	217	453	Re-Rx, new drug			
11918	10.0	5	98	52	320	222	617	887	Re-Rx, new drug			
12314	10.0	5	88	40	124	84	19	45	Re-Rx, new drug			
12194	50.0	1	36	36	345	142	394	241	Re-Rx, new drug			
12195	50.0	4	88	75	567	395	850	813	Re-Rx, new drug			
12242	2.5a)	10	102	40	517	429	551	641	Re-Rx, higher dose			
12242r	2.5a)	641	395	120	Died - malaria							

a) Pyrimethamine

TABLE 5

SUMMARY OF THE ACTIVITY OF WR 003019AM (BL 18309), PROGUANIL, AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12197	1.0	+			n.a.	n.a.	Re-Rx, new drug
12326	1.0	+			n.a.	n.a.	Re-Rx, new drug
11918	10.0	+			n.a.	n.a.	Re-Rx, new drug
12314	10.0	+			n.a.	n.a.	Re-Rx, new drug
12194	50.0	+			n.a.	n.a.	Re-Rx, new drug
12195	50.0	+			n.a.	n.a.	Re-Rx, new drug
12242	2.5a	+			n.a.	n.a.	Re-Rx, same dose
12242r	2.5a	+			n.a.	n.a.	Died, malaria, Day 3 of Rx

a. Pyrimethamine

TABLE 6

ACTIVITY OF WR 003019AM (BL 18309), PROGUANIL, AGAINST PLASMODIUM FALCIPARUM INFECTIONS

MALARIA STRAIN	DOSE mg/kg TOTAL	DOSE mg/kg DAILY	PRIMARY TREATMENTS CLEARED	PRIMARY TREATMENTS CURED	REPEAT TREATMENTS CLEARED	REPEAT TREATMENTS CURED	TOTAL TREATMENTS CLEARED	TOTAL TREATMENTS CURED
Smith	3.0	1.0	0/2	0/2			0/2	0/2
	30.0	10.0	0/2	0/2			0/2	0/2
	150.0	50.0	0/2	0/2			0/2	0/2

TABLE 7

DETAILED ACTIVITY OF WR 250417AA (BK 47734), PROGUANIL ANALOG, AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12039r	0.1	296	517	493	172	Died - malaria						
12174r	0.1	57	490	67	284	265	290	345	196	Re-Rx, higher dose		
12197r	0.1	517	453	296	222	Died - malaria						
12314r	0.1	45	81	78	64	82	228	111	72	Re-Rx, higher dose		
12183	1.0	1	51	9	13	2	0.06	<0.01	0	0	0	0
12214	1.0	3	80	48	50	1	0.05	<0.01	0	0	0	0
12138r	1.0	28	45	10	6	0.06	<0.01	0	0	0	0	0
12165r	1.0	50	80	74	7	51	0.4	<0.01	<0.01	<0.01	0	0
12175r	1.0	<0.01	0.8	0.5	0.07	<0.01	0	0	0	0	0	0
12326r	1.0	453	429	586	308	3	<0.01	0	0	0	0	0
12174rr	1.0	196	300	301	66	50	1	0.06	<0.01	0	0	0
12314rr	1.0	72	57	40	0.7	<0.01	0	0	0	0	Died a)	
11935	10.0	3	51	22	14	7	0.2	<0.01	0	0	0	0
12216	10.0	3	99	29	19	1	<0.01	0	0	<0.01	0	0
11913r	10.0	887	690	405	160	57	13	0.6	0.3	<0.01	0	0
12168r	10.0	593	791	838	Died, malaria							
12194r	10.0	241	590	117	45	2	<0.01	0	0	0	0	0
12195r	10.0	813	1059	198	Died, malaria							
12214r	10.0	1	30	21	5	0.2	<0.01	<0.01	0.1	<0.01	<0.01	0
12165rr	10.0	0.6	2	0.6	<0.01	0		0	0	0	0	0
12175rr	10.0	<0.01	<0.01	<0.01	<0.01	0	0	0	0	0	0	0
12326rr	10.0	<0.01	1	0.1	<0.01	<0.01	0	0	0	0	0	0
							0					
11928	50.0	9	82	74	34	15	0.6	<0.01	0	0	0	0
12215	50.0	0.8	16	20	15	0.8	<0.01	<0.01	<0.01	0	0	0
12214rr	50.0	4	1	10	1	1	2	0.1	0	0	0	0
12165rrr	50.0	0.6	0.8	<0.01	<0.01	0	0	0	0	0	0	0

a) Intestinal obstruction

TABLE 7 (CONT'D)

DETAILED ACTIVITY OF WR 250417AA (BK 47734), PROGUANIL ANALOG, AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12242	2.5b)	10	102	40	517	429	551	641	Re-Rx, higher dose			
12242	2.5b)	641	395	120	Died, malaria							

b) Pyrimethamine

TABLE 8

SUMMARY OF THE ACTIVITY OF WR 250417AA (BK 47734), PROGUANIL ANALOG, AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12039r	0.1	+			n.a.	n.a.	Died, malaria, Day 1-Post-Rx
12174r	0.1	+			n.a.	n.a.	Re-Rx, higher dose
12197r	0.1	+			n.a.	n.a.	Died, malaria, Day 1-Post-Rx
12314r	0.1		+		n.a.	n.a.	Re-Rx, higher dose
12183	1.0			+	7	n.a	Cured
12214	1.0			+	7	17	Re-Rx, higher dose
12138r	1.0			+	6	n.a.	Cured
12165r	1.0			+	9	16	Re-Rx, higher dose
12175r	1.0			+	5	18	Re-Rx, higher dose
12326r	1.0			+	6	32	Re-Rx, higher dose
12174rr	1.0			+	8	n.a.	Cured
12314rr	1.0			+	5	n.a.	4, Died Day 6-Post-Rx a)
11935	10.0			+	7	n.a.	Cured
12216	10.0			+	6	n.a.	Cured
11918r	10.0			+	9	n.a.	Cured
12168r	10.0				n.a.	n.a.	Died, malaria, Day 3 of Rx
12194r	10.0			+	6	n.a.	Cured
12195r	10.0	+			n.a.	n.a.	Died, malaria, Day 3 of Rx
12214r	10.0			+	10	17	Re-Rx, higher dose
12165rr	10.0			+	4	21	Re-Rx, higher dose
12175rr	10.0			+	4	n.a.	Cured
12326rr	10.0			+	4	n.a.	Cured
11928	50.0			+	7	n.a.	Cured
12215	50.0			+	8	n.a.	Cured
12214rr	50.0			+	7	n.a.	Cured

a) Intestinal Obstruction

TABLE 8 (CONT'D)

SUMMARY OF THE ACTIVITY OF WR 250417AA (BK 47734), PROGUANIL ANALOG, AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM (CONT'D)

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12165rrr	50.0			+	4	n.a.	Cured
12242r	2.5b	+			n.a.	n.a.	Died, malaria, Day 3 of Rx

b) Pyrimethamine

TABLE 9

ACTIVITY OF WR 250417AA (BK 47734), PROGUANIL ANALOG, AGAINST INFECTIONS OF PLASMODIUM FALCIPARUM

MALARIA STRAIN	DOSE mg/kg TOTAL	DOSE mg/kg DAILY	PRIMARY TREATMENTS CLEARED	PRIMARY TREATMENTS CURED	REPEAT TREATMENTS CLEARED	REPEAT TREATMENTS CURED	TOTAL TREATMENTS CLEARED	TOTAL TREATMENTS CURED
Smith	0.3	0.1			0/4	0/4	0/4	0/4
	3.0	1.0	2/2	1/2	6/6	2/5	8/8	3/7
	30.0	10.0	2/2	2/2	6/6	4/6	8/8	6/8
	150.0	50.0	2/2	2/2	2/2	2/2	4/4	4/4

IN VIVO TRIALS TO REVERSE CHLOROQUINE RESISTANCE OF *PLASMODIUM FALCIPARUM* BY THE CONCOMITANT ADMINISTRATION OF CALCIUM CHANNEL BLOCKERS OR SIMILAR ACTING DRUGS PLUS CHLOROQUINE

A. Introduction

Subsequent to the initial reports (1, 2) of chloroquine resistance in human *P. falciparum*, intensive efforts have been focused upon the identification of new drugs effective against such strains and, to a much, lesser extent, the mechanism of chloroquine resistance. Two recent reports (3,4) indicate the *in vitro* reversal of chloroquine resistance by the use of verapamil, a calcium channel blocker. To explain this observation, it is proposed that chloroquine-resistant falciparum parasites prevent the accumulation of chloroquine in the infected erythrocyte and thus escape the cytocidal action of the drug. The use of a calcium channel blocker, or other similar acting drugs, stops the active efflux of chloroquine which then accumulates to a toxic level and kills the parasite. Based upon the *in vitro* observations, a series of trials were initiated to determine if *in vivo* reversal of chloroquine-resistance is feasible, using the *Aotus* model. If such were possible in the monkey model, and subsequent human trials substantiated these results, then treatment of patients with chloroquine-resistant *P. falciparum* infections would be significantly enhanced.

B. Response of infections of the Vietnam Smith strain of P. falciparum to chloroquine (WR 001544BM: BN:AR 20613)

The detailed response of the parasitemias in chloroquine only treated Aotus is presented in the tables for each of the experiments in this section. The results are summarized in Table 10 and indicate that primary treatments with chloroquine did not clear parasitemias, although retreatments did effect parasite clearance. No infections of the chloroquine-resistant Smith strain were cured by chloroquine alone.

During primary treatment with chloroquine, a suppression of parasitemia, in comparison with the untreated controls, was observed. Since the Smith strain was identified (5) as being RIII resistant to chloroquine, there may have been the possibility that passage of parasites by infected blood for more than 12 years and occasional cryopreservation and thawing of the parasites may have resulted in the selection of a strain with increased sensitivity to chloroquine. As described in the next section, a strain of Smith parasites was isolated with a projected higher resistance to chloroquine.

TABLE 10

ACTIVITY OF CHLOROQUINE (WR 001544BM; BN: AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

MALARIA	DOSE mg/kg		PRIMARY TREATMENTS		REPEAT TREATMENTS		TOTAL TREATMENTS	
STRAIN	TOTAL	DAILY	CLEARED	CURED	CLEARED	CURED	CLEARED	CURED
Smith	60.0	20.0	0/3	0/3	1/1	0/1	1/4	0/4
	80.0	40/20/20*	0/2	0/2	2/2	0/2	2/4	0/4

* Administered as 40.0 mg/kg on Day 1, and 20.0 mg/kg on Days 2 and 3

C. Isolation of the Vietnam Smith/RE strain of P. falciparum and its response to chloroquine (WR 001544BM; BN:AR 20613)

Aotus 12328, was used as a chloroquine treated control in experiments described in a subsequent section. Primary treatment was 40.0 mg per kg of chloroquine on day 1, and 20.0 mg per kg on days 2 and 3.

There was suppression of parasitemia, and beginning on day 5 after termination of the initial regimen, a second course of treatment was initiated at the same doses. The parasitemia was cleared on day 12 after the termination of the second regimen, with a recrudescence beginning on day 34 after treatment.

Parasites, during this recrudescence, were subinoculated into each of three Aotus, and evaluated for their response to chloroquine (Tables 11, 12, and 13). The data in these tables show that primary treatment, with either 20.0 mg base per kg (x 3 days), or 40.0 mg base per kg (x 1 day), and 20.0 mg per kg (x 2 days), suppressed the parasitemia. Retreatment with the 20.0 mg per kg (x 3) cured the infection, while retreatment with the second regimen cleared the parasitemia, but did not cure the infection.

This strain was called the Vietnam Smith/RE and used for evaluation of some calcium channel blockers, as detailed in succeeding sections.

TABLE 11

DETAILED ACTIVITY OF WR 001544BM (AR 20613), CHLOROQUINE, AGAINST RECRUDESCENT PARASITES OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
11020	20.0	4	13	57	29	51	130	111	161	Re-Rx, same dose		
12006	40/20a	4	10	14	28	0.8	3	28	71	Re-Rx, same dose		
11020r	20.0	161	97	54	95	837	33	11	13	2	0.2	<0.01
12006r	40/20a	71	72	142	81	71	56	12	6	1	0.08	<0.01

a. 40 mg/kg day 1, 20.0 mg/kg days 2 and 3.

TABLE 12

SUMMARY OF THE EVALUATION OF CHLOROQUINE (WR 001544BM; AR 20613)
AGAINST RECRUDESCENT PARASITES OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Note
		None	Suppressed	Cleared			
11020	20.0		+		n.a.	n.a.	Re-Rx, same dose
12006	40/20a		+		n.a.	n.a.	Re-Rx, same dose
11020r	20.0			+	11	n.a.	Cured
12006r	40/20a			+	14	42	

a. 40.0 mg/kg day 1, 20.0 mg/kg days 2 and 3.

TABLE 13

ACTIVITY OF CHLOROQUINE (WR 001544BM; BN: AR 20613) AGAINST RECRUDESCENT PARASITES OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

MALARIA	DOSE mg/kg		PRIMARY TREATMENTS		REPEAT TREATMENTS		TOTAL TREATMENTS	
STRAIN	TOTAL	DAILY	CLEARED	CURED	CLEARED	CURED	CLEARED	CURED
Smith	60.0	20.0	0/1	0/1	1/1	1/1	1/2	1/2
	80.0	40.0x1 20.0x2	0/1	0/1	1/1	0/1	1/2	0/2

D. WR 255694AB; BN:BL 22009 - Verapamil

Detailed results of trials with verapamil to reverse chloroquine resistance of infections with the Vietnam Smith strain are shown in Table 14, and summarized in Table 15 and 17. Evaluation of verapamil alone indicated that parasitemia was cleared in each of two Aotus subsequent to a 3-day dosage of 25.0 mg per kg. Reference to the detailed course of parasitemia in Table 14, shows that the parasitemia in Aotus 11806 was suppressed, but not suppressed in Aotus 12010. The rate of parasite clearance in these Aotus was equal to that which occurs in untreated Vietnam Smith infections and, therefore, cannot be wholly attributable to drug action. A 3-day dosage of 50.0 and 100.0 mg per kg had no effect upon parasitemias in a total of three monkeys, and all died of apparent drug toxicity on days 5, 7, and 3 after the completion of treatment.

Each of two Aotus received a 3-day dosage of 25.0 mg per kg of verapamil plus 20.0 mg per kg of chloroquine. Vietnam Smith parasitemias were cleared in both monkeys, but again at a rate similar to that seen in untreated infections. The infection in Aotus 11995 was cured while Aotus 12000 died of an intercurrent infection 29 days after the end of treatment. A 3-day dosage of 50.0 mg per kg of verapamil plus 20.0 mg per kg of chloroquine had no activity against the parasitemia in each of two Aotus; one monkey died of malaria on day 5 after the end of treatment, and the other animal died on day 2 of treatment, probably of drug toxicity.

Chloroquine, alone, at a 3-day dosage of 20.0 mg per kg had some suppressive effect upon the parasitemia, but both animals succumbed to the malaria infection.

Results of the evaluation of verapamil and chloroquine against Vietnam Smith/RE infections are presented in Tables 14, 16, and 17. For this study, verapamil was administered three times per day (8:00 AM, Noon, 4:00 PM), in an attempt to maintain a high blood concentration of the calcium channel blocker. A 3-day dosage of 5.0 mg per kg of verapamil alone had no activity against parasitemia, and retreatment with 10.0 mg per kg of verapamil plus a single daily dosage of 20.0 mg per kg was not effective, as the animal died of malaria on day 2 of treatment.

Treatment with a 3-day dosage of verapamil at 5.0 mg per kg plus a daily dose of 20.0 mg per kg of chloroquine has no activity against the parasitemia in one Aotus, and suppressed the parasitemia in Aotus 11940, that died on day 2 retreatment with 10.0 mg per kg of verapamil plus 20.0 mg per kg of chloroquine.

A 3-day dosage of 20.0 mg per kg of chloroquine had no effect upon parasitemia in Aotus 11921, that died of malaria on day 4 post treatment. This dosage of chloroquine suppressed the parasitemia in 11932, and retreatment with the same dosage cured the infection.

TABLE 14

DETAILED ACTIVITY OF WR 255694AB (BL 22009), VERAPAMIL, ALONE AND IN COMBINATION WITH WR 001544BM (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
						VIETNAM SMITH STRAIN						
11806	25.0a	8	120	57	231	231	329	129	217	213	302	219
12010	25.0a	8	220	131	817	1025	506	346	96	135	110	120
11371	50.0a	5	151	51	302	408	746	568	498	Died-drug toxicity		
12295	50.0a	11	128	51	471	294	745	426	321	535	328	Died*
12042	100.0a	5	69	31	497	Died-drug toxicity						
11994	20.0b	6	100	78	82	48	115	76	Died-drug toxicity			
12041	20.0b	10	127	20	169	87	98	64	38	198	87	284
11995	25.0a 20.0b	5	100	17	129	27	40	20	135	162	211	220
12000	25.0a 20.0b	4	47	42	158	102	310	137	216	204	471	248
12287	50.0a 20.0b	8	104	117	560	302	507	1089	449	622	Died-malaria	
12292	50.0a 20.0b	5	87	47	Died-drug toxicity							

* Drug toxicity
a Verapamil
b Chloroquine.

TABLE 14 (CONT'D)

DETAILED ACTIVITY OF WR 255694AB (BL 22009), VERAPAMIL, ALONE AND IN COMBINATION WITH WR 001544BM (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
						VIETNAM SMITH/RE STRAIN						
12205	5.0a	20	40	53	234	222	537	444	326	671	Re-Rx	
11919	5.0a 20.0b	26	83	103	102	370	567	798	542	739	Died-malaria	
11940	5.0a 20.0b	17	60	95	33	25	49	74	259	203	Re-Rx, higher dose	
12205r	10.0a 20.0	671	197	Died-malaria								
11940r	10.0a 20.0b	203	179	Died-malaria								
11921	20.0b	16	71	100	221	418	961	887	Died-malaria			
11932	20.0b	14	49	74	53	152	136	99	19	130	Re-Rx, same dose	
11932r	20.0b	130	136	111	79	86	49	111	4	0.9	<0.01	0

a Verapamil - 3x daily
b Chloroquine - daily dose

TABLE 15

SUMMARY OF THE ACTIVITY OF VERAPAMIL (WR 255694AB; BL 22009), ALONE, AND IN COMBINATION WITH CHLOROQUINE (WR 001544; BM AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
11806	25.0a			+	21	53	
12010	25.0a			+	20	7	
11371	50.0a	+					
12295	50.0a	+			n.a.	n.a.	Died Day 5 Post-Rx*
					n.a.	n.a.	Died Day 7 Post-Rx*
12042	100.0a	+					
					n.a.	n.a.	Died Day 3 Post-Rx*
11994	20.0b						
12041	20.0b		±		n.a.	n.a.	Died Day 4 Post-Rx*?
			+		n.a.	n.a.	Died Day 20 Post-Rx-malaria
11995	25.0a						
	20.0b			+	21	n.a.	Cured
12000	25.0a						
	20.0b			+	19	n.a.	Died Day 29 Post-Rx Intercurrent Infection
12287	50.0a	+					
	20.0b				n.a.	n.a.	Died Day 5 Post-Rx, malaria
12292	50.0a	+					
	20.0b				n.a.	n.a.	Died Day 2 of Rx*

a Verapamil
b Chloroquine
* Drug toxicity

TABLE 16

SUMMARY OF THE ACTIVITY OF VERAPAMIL (WR 255694AB: BL 22009) PLUS CHLOROQUINE (WR 1544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
12205	5.0a	+			n.a.	n.a.	Re-Rx, higher dose+chloroquine
11919	5.0a 20.0b	+			n.a.	n.a.	Died Day 6 post Rx, malaria
11940	5.0a 20.0b		+		n.a.	n.a.	Re-Rx, higher dose
12205r	10.0a 20.0b		n.a.		n.a.	n.a.	Died Day 2 Rx, malaria
11940r	10.0a 20.0b		n.a.		n.a.	n.a.	Died 2 Rx, malaria
11921	20.0b	+			n.a.	n.a.	Died Day 4 Post Rx, Malaria
11932	20.0b		+		n.a.	n.a.	Re-Rx, same dose
11932r	20.0b			+	10	n.a.	Cured

a. Verapamil 3 x/day
b. Chloroquine - daily dose

TABLE 17

ACTIVITY OF VERAPAMIL (WR 255694AB; BN:BL 22009), ALONE, AND IN COMBINATION WITH CHLOROQUINE (WR 001544BM; BN:AR 20613) AGAINST INFECTIONS OF TWO STRAINS OF PLASMODIUM FALCIPARUM

MALARIA	DOSE mg/kg		PRIMARY TREATMENTS		REPEAT TREATMENTS		TOTAL TREATMENTS	
STRAIN	TOTAL	DAILY	CLEARED	CURED	CLEARED	CURED	CLEARED	CURED
VIETNAM SMITH	75.0	25.0a	2/2	0/2			2/2	0/2
	150.0	50.0a	0/2	0/2			0/2	0/2
	300.0	100.0a	0/1	0/1			0/1	0/1
	75.0 60.0	25.0a 20.0b	2/2	1/2			2/2	1/2
	150.0 60.0	50.0a 20.0b	0/2	0/2			0/2	0/2
	60.0	20.0b	0/2	0/2			0/2	0/2
SMITH/RE	45.0	15.0aa	0/1	0/1			0/1	0/1
	45.0 60.0	15.0aa 20.0b	0/2	0/2			0/2	0/2
	60.0	20.0b	0/2	0/1	1/1	1/1	1/3	1/2

a Verapamil
aa Verapamil 5.0 mg/kg 3x/day
b Chloroquine

E. WR 255695AC; BN:BL 21995 - Nifedipine

Nifedipine, a calcium channel blocker, was used in trials to reverse chloroquine-resistance of Vietnam Smith infections (Tables 18, 19, 20, 21, and 26) As presented in Tables 18 and 19, a 3-day dosage of nifedipine alone of 5.0 mg per kg had no activity against the parasitemia, nor did retreatment with a 3-day dosage of 20.0 mg per kg of nifedipine plus 20.0 mg per kg of chloroquine. The animal died of malaria on day 2 post treatment.

Nifedipine, alone, administered in a 3-day dosage of 10.0 mg per kg was inactive against Smith parasites. Retreatment with a 3-day dosage of 40.0 mg per kg of nifedipine plus 20.0 mg per kg of chloroquine cured the infection.

Nifedipine administered for three days at 5.0 mg per kg plus 20.0 mg per kg chloroquine suppressed the parasitemia in one Aotus, and retreatment with a 3-day dosage of 20.0 mg per kg of nifedipine plus 20.0 mg per kg of chloroquine cured the infection. A primary treatment of nifedipine at 10.0 mg per kg for 3 days plus chloroquine at 20.0 mg per kg for 3 days suppressed parasitemia in one monkey. Retreatment with nifedipine at 40.0 mg per kg for 3 days cured the infection.

The parasitemia in one chloroquine - only treated monkey was suppressed by a 3-day dosage of 20.0 mg per kg, and a repeat of this regimen cleared the parasitemia, but did not cure the infection, as indicated by a recrudescence.

Results of a second trial to reverse chloroquine-resistance of the Vietnam Smith strain with nefedipine and chloroquine are shown on Tables 20 and 21. In this experiment, nifedipine was administered three times per day, at 4 hour intervals starting at 8:00 AM, for three days. Chloroquine was administered on day 1 of treatment at 40.0 mg per kg, and on days 2 and 3 at 20.0 mg per kg. The hypothesis underlying this increase of frequency of administering nifedipine and a loading dose of chloroquine on day 1 of treatment, might increase the drug blood levels and achieve reversal of resistance to chloroquine.

The parasitemia in each of two Aotus administered nifedipine at 5.0 mg per kg for days was not affected and both animals died of malaria on post treatment days 4, and 5, respectively.

Two Aotus each received nifedipine in a 3-day dosage of 5.0 mg per kg (3x/day) plus chloroquine (40.0, 20.0,20.0 mg per kg). Parasitemias were suppressed in both monkeys and retreated with 10.0 mg per kg of nifedipine and chloroquine, as in the primary treatment. This retreatment cleared parasitemias in both monkeys, and cured the infection in one animal.

Two Aotus served as chloroquine only treated controls. The

primary treatment with a 3-day dosage of 40.0, 20.0, and 20.0 mg per kg suppressed the parasitemia in each of the two monkeys, and retreatment with the same regimen cleared parasitemias, but did not cure the infection.

Nifedipine plus chloroquine was evaluated for its potential to reverse chloroquine resistance of Vietnam Smith/RE infections. The results are presented in Tables 22-25, and summarized in Tables 26. Nifedipine administered 3x/day at 5.0 mg per kg for three days plus chloroquine for three days at 20.0 mg per kg daily had no effect upon the parasitemia in Aotus 11943, and died of malaria on day 5 post treatment. The parasitemia was suppressed in Aotus 11946 by the same dosages and retreatment with 10.0 mg per kg (3x/day) of nifedipine for three days plus chloroquine had no antimalarial activity. The animal died of an intercurrent infection on day 9 post treatment.

One monkey died of malaria after a 3-day dosage of 20.0 mg per kg of chloroquine; this dosage suppressed the parasitemia in another monkey and cured the infection following re-treatment.

The results shown in Tables 24 and 25 are those from a trial in which it was planned to administer 20.0 mg per kg of nifedipine three times per day for seven days plus 20.0 mg per kg of chloroquine for treatment days 1, 2 and 3. Aotus 11916 died of malaria on day 5 of the treatment period, and Aotus 11917 died of malaria on day 7, after having received the last drug dose.

A three day treatment with 20.0 mg per kg of chloroquine showed the parasites refractory to the drug and the animal died of malaria on day 4 post treatment.

TABLE 18

DETAILED ACTIVITY OF NIFEDIPINE (WR 255695AC; BL 21995), ALONE, AND IN COMBINATION WITH CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12156	5.0a	2	37	109	140	429	342	923	Re-Rx, higher dose plus CQ			
12156r	20.0a	923	568	945	491	389	Died- malaria					
	20.0b											
12155	10.0a	1	7	29	284	338	372	882	Re-Rx, higher dose plus CQ			
12155r	40.0a	882	745	1030	480	215	132	82	70	10	4	0.7
	20.0b											
12121	5.0a	0.4	19	51	142	100	142	290	Re-Rx, higher dose			
	20.0b											
12121r	20.0a	290	335	284	240	92	75	36	20	14	5	0.6
	20.0b											
12120	10.0a	0.5	18	44	72	40	6	30	Re-Rx, higher dose			
	20.0b											
12121r	40.0a	30	186	134	204	80	138	72	81	29	35	34
	20.0b											
11373	20.0b	1	4	15	110	113	213	284	Re-Rx, same dose			
11373r	20.0b	284	373	266	212	95	60	20	82	14	9	5

a. Nifedipine
b. Chloroquine = CQ

TABLE 19

SUMMARY OF THE ACTIVITY OF NIFEDIPINE (WR 255695AC; BL 21195), ALONE AND IN COMBINATION WITH CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12156	5.0a	+			n.a.	n.a.	Re-Rx, higher dose plus CQ
12156r	20.0a 20.0b	+			n.a.	n.a.	Died Day 2, Post-Rx
12155	10.0a	+			n.a.	n.a.	Re-Rx, higher dose plus CQ
12155r	40.0a 20.0b			+	11	n.a.	Cured
12121	5.0a 20.0b		+		n.a.	n.a.	Re-Rx, Higher dose
12121r	20.0a 20.0b			+	11	n.a.	Cured
12120	10.0a 20.0b		+		n.a.	n.a.	Re-Rx, higher dose
12120r	40.0a 20.0b			+	12	n.a.	Cured
11373	20.0b		±	+	n.a.	n.a.	Re-Rx, same dose
11373r	20.0b				10	33	

a= Nifedipine
b= Chloroquine = CQ

TABLE 20

DETAILED ACTIVITY OF NIFEDIPINE (WR 255695AC; BL 21995), ALONE, AND IN COMBINATION WITH CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM-SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12285	5.0a	10	120	55	321	214	296	370	388	Died - malaria		
12288	5.0a	6	57	46	517	346	241	902	622	Died - malaria		
11437	5.0a 40/20b	4	65	20	36	25	345	391	592	Re-Rx, higher dose		
12072	5.0a 40/20b	2	41	11	8	3	14	5	29	Re-Rx, higher dose		
11437r	10.0a 40/20b	592	715	321	581	55	311	228	73	9	3	1
12072r	10.0a 40/20b	29	6	14	5	21	12	4	1	4	0.2	3
12328	40/20b	10	99	82	15	1	12	30	161	Re-Rx, same dose		
12329	40/20b	10	112	73	0.6	<0.01	<0.01	<0.01	0.6	Re-Rx, same dose		
12328r	40/20b	161	221	142	87	11	29	2	3	3	3	26
12329r	40/20b	0.6	0.8	2	0.1	0.6	2	0.4	0.6	0.2	0.3	<0.01

a Nifedipine 3x/day
b Chloroquine 40.0 mg/kg Day 1, 20.0 mg/kg Day 2 and 3

TABLE 21

SUMMARY OF THE ACTIVITY OF NIFEDIPINE (WR 255695AC; BL 21995), ALONE, AND IN COMBINATION WITH CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/kg	Response of Parasitemia to Rx			Days from Initial Rx. to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12285	5.0a		±		n.a.	n.a.	Died Day 4 Post-Rx,malaria
12288	5.0a.	+			n.a.	n.a.	Died Day 5 Post-Rx,malaria
11437	5.0a 40/20b		±		n.a.	n.a.	Re-Rx, higher dose
12072	5.0 40/20b		+		n.a.	n.a.	Re-Rx, higher dose
11437r	10.0a 40/20b			+	14	46	
12072r	10.0a 40/20b			+	14	n.a.	Cured
12328	40/20b		+		n.a.	n.a.	Re-Rx, same dose
12329	40/20b		+		n.a.	n.a.	Re-Rx, same dose
12328r	40/20b			+	15	34	
12329r	40/20b			+	14	27	

a. Nifedipine 3 x/day
b. Chloroquine 40.0 mg/kg Day 1, 20.0 mg/kg Day 2 and 3.

TABLE 22

DETAILED ACTIVITY OF NIFEDIPINE (WR 255695AC; BL 21995) PLUS CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
11943	5.0a 20.0b	20	70	60	288	161	690	788	167	Died - malaria		
11946	5.0a 20.0b	10	35	56	80	130	71	87	184	142	Re-Rx, higher dose	
11946r	10.0a 20.0b	142	234	259	204	160	113	99	20	1	0.2	< 0.01
11921	20.0b	16	71	100	221	418	961	887	Died - malaria			
11932	20.0b	14	49	74	55	152	136	99	19	130	Re-Rx, same dose	
11932r	20.0b	130	136	111	79	86	49	111	4	0.9	< 0.01	0

a Nifedipine 3x/day
b Chloroquine daily dose

TABLE 23

SUMMARY OF THE ACTIVITY OF NIFEDIPINE (WR 255695AC; BL 21995) PLUS CHLOROQUINE (WR 1544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/kg	Response of Parasitemia to Rx			Days from Initial Rx. to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
11943	5.0a 20.0b	+			n.a.	n.a.	Died Day 5 Post Rx,Malaria
11946	5.0a 20.0b		+		n.a.	n.a.	Re-Rx, higher dose
11946r	10.0a 20.0b			n.a.	n.a.	n.a.	Died Day 9 Post-Rx*
11921	20.0b	+			n.a.	n.a.	Died Day 4 Post Rx,Malaria
11932	20.0b		+		n.a.	n.a.	Re-Rx, same dose
11932r	20.0b			+	10	n.a.	Cured

a. Nifedipine, 3x/day
b. Chloroquine, daily dose
* Intercurrent infection

TABLE 24

DETAILED ACTIVITY OF NIFEDIPINE (WR 255695AC; BL 21995) PLUS CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3									
		Day Pre-Rx	Day of Treatment							Day Post Treatment		
			1	2	3	4	5	6	7	1	2	3
11916	20.0a 20.0b	25	209	468	321	388	Died-malaria					
11917	20.0a 20.0b	18	234	345	367	321	467	542	357	Died- malaria		
12178	20.0b	6	160	265	284	542	517	813	Died - malaria			

a Nifedipine - 3x day
b Chloroquine - 1x/day, on treatment days 1,2 and 3 only

TABLE 25

SUMMARY OF THE ACTIVITY OF NIFEDIPINE (WR 255695AC; BL 21995) PLUS CHLOROQUINE, WR 001544BM (AR 20613), AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 7 Mg/kg	Response of Parasitemia to Rx			Days from Initial Rx. to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
11916	20.0a 20.0b	+			n.a.	n.a.	Died day 5 of Rx, malaria
11917	20.0a 20.0b	+			n.a.	n.a.	Died by 7 of Rx, malaria
12178	20.0b	+			n.a.	n.a.	Died day 4 post Rx, malaria

a. WR 255695 - 3 x/day

b. Chloroquine - 1 x/day, treatment days 1-2-3

TABLE 26

ACTIVITY OF NIFEDIPINE (WR 255695AC; BN:BL 21995), ALONE AND IN COMBINATION WITH CHLOROQUINE (WR 00154 BM; BN:AR 20613) AGAINST INFECTIONS OF TWO STRAINS OF PLASMODIUM FALCIPARUM

MALARIA STRAIN	DOSE mg/kg TOTAL	DOSE mg/kg DAILY	PRIMARY TREATMENTS CLEARED	PRIMARY TREATMENTS CURED	REPEAT TREATMENTS CLEARED	REPEAT TREATMENTS CURED	TOTAL TREATMENTS CLEARED	TOTAL TREATMENTS CURED
VIETNAM SMITH	15.0	5.0a	0/3	0/3			0/3	0/3
	30.0	10.0a	0/1	0/1			0/1	0/1
	15.0 60.0	5.0a 20.0b	0/1	0/1			0/1	0/1
	30.0 60.0	10.0a 20.0b	0/1	0/1			0/1	0/1
	60.0 60.0	20.0a 20.0b			1/2	1/1	1/2	1/1
	120.0 60.0	40.0a 20.0b			2/2	2/2	2/2	2/2
	15.0 80.0	5.0a 40/20/20bb	0/2	0/2			0/2	0/2
	30.0 80.0	10.0a 40/20/20bb			2/2	1/2	2/2	1/2
	60.0	20.0b	0/1	0/1	1/1	0/1	1/2	0/2
	80.0	40/20/20bb	0/2	0/2	2/2	0/2	2/4	0/4
SMITH/RE	45.0 60.0	15.0a 20.0b	0/2	0/2			0/2	0/2
	90.0 60.0	30.0a 20.0b			0/1	0/1	0/1	0/1
	420.0 60.0	60.0a 20.0b	0/2	0/2			0/2	0/2
	60.0	20.0b	0/3	0/3	1/1	1/1	1/4	1/4

a. Nifedipine
b. Chloroquine
bb. Chloroquine 40.0 mg/kg Day 1, 20.0 mg/kg Days 2 and 3

F. WR 256287AA; BN:BL 28636
WR 256287AB; BN:BL 51153

This drug, prepared by Hoffman La Roche, is a structural analog of verapamil and is putatively more active as a calcium channel blocker in humans. It is also 4x more effective than verapamil in reversing chloroquine resistance in vitro. Two chemically identical lots of WR 256287 were used for in vivo trials. Results of the initial study against infections of the Vietnam Smith strain are shown in Tables 27 and 28.

A 3-day dosage of 1.0 mg per kg of WR 256287 alone showed no effect upon parasitemia. Retreatment with 5.0 mg per kg of the experimental drug plus 20.0 mg per kg of chloroquine for three days cleared the parasitemia, but did not cure the infection.

WR 256287, alone, administered for three days at 25.0 mg per kg had no activity on the parasitemia. Retreatment with WR 256287 at 50.0 mg per kg plus chloroquine at 20.0 mg per kg, both for three days, cleared the parasitemia, but a recrudescence occurred.

Primary treatment for three days WR 256287, at 1.0 or 25.0 mg per kg, each plus 20.0 mg per kg of chloroquine, did not effect the parasitemia. Retreatment with WR 256287 for three days at 5.0 or 50.0 mg per kg, plus chloroquine at 20.0 mg per kg, for each dose, cured the infection in 2 of 2 Aotus.

Primary treatment with chloroquine only at 20.0 mg per kg (x 3 days) had a questionable suppressive effect. Retreatment with this dosage cleared the parasitemia, but did not cure the infection.

Data associated with trial to reverse chloroquine resistance in Vietnam Smith/RE infections are shown in Tables 29, 30, 31, and summarized in Table 32. In each of two Aotus, the primary treatment consisted of WR 256287 at 20,0 mg per kg administered three time daily for seven days plus chloroquine at 20.0 mg per kg administered for the first three days of the treatment period. Parasitemias in both monkeys were suppressed significantly in comparison with the parasitemia in the chloroquine only treated monkey.

Retreatment in both monkeys with a 3-day dosage consisted of WR 256287 at 50.0 mg per kg once a day plus chloroquine daily at 20.0 mg per kg. Parasitemias were cleared, but the infections were not cured.

TABLE 27

DETAILED ACTIVITY OF 256287AA (BL 28636), ALONE, AND IN COMBINATION WITH CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
11937	1.0a	3	40	53	231	408	512	427	Re-Rx, higher dose plus CQ			
11937r	5.0a	427	726	408	251	225	201	21	12	4	1	3
	20.0b											
11968	25.0a	2	19	40	186	302	408	312	Re-Rx, higher dose plus CQ			
11968r	50.0a	312	306	355	146	116	119	77	71	24	33	30
	20.0b											
11504	1.0a	1	8	20	124	70	371	260	Re-Rx, higher dose			
	20.0b											
11504r	5.0a	260	949	293	160	97	39	19	18	1	14	3
	20.0b											
11304	25.0a	0.6	9	29	142	58	124	222	Re-Rx, higher dose			
	20.0b											
11304r	50.0a	222	409	364	168	216	157	80	64	19	12	12
	20.0b											
11373	20.0b	1	4	15	110	113	213	284	373	Re-Rx, same dose		
11373rb	20.0b	284	373	266	212	95	60	20	82	14	9	5

a= WR 256287
b= Chloroquine= CQ

TABLE 28

SUMMARY OF THE ACTIVITY OF WR 256287AA (BL 28636), ALONE, AND IN COMBINATION WITH CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
11937	1.0a	+			n.a.	n.a.	
11937r	5.0a 20.0b			+	12	25	Re-Rx, higher dose plus CQ
11968	25.0a	+			n.a.	n.a.	
11968r	50.0a 20.0b			+	12	31	Re-Rx, higher dose plus CQ
11504	1.0a 20.0b	+			n.a.	n.a.	Re-Rx, higher dose
11504r	5.0a 20.0b			+	10	n.a.	Cured
11304	25.0a 20.0b		±		n.a.	n.a.	Re-Rx, higher dose
11304r	50.0a 20.0b			+	12	n.a.	Cured
11373	20.0b		±		n.a.	n.a.	
11373r	20.0b			+	10	33	Re-Rx, same dose

a= WR 256287
b= Chloroquine = CQ

TABLE 29

DETAILED ACTIVITY OF WR 256287AA (BL 28636) /WR 256287AB (BL 51153 PLUS CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3									
		Day Pre-Rx	Day of Treatment							Day Post Treatment		
			1	2	3	4	5	6	7	1	2	3
11969	20.0a 20.0b	11	167	65	87	53	2	4	14	65	Re-Rx, Table 30	
12204	20.0a 20.0b	14	204	105	19	31	5	2	65	58	Re-Rx, Table 30	
12178	20.0b	6	160	265	284	542	517	813	Died-malaria			

a WR 256287 - 3x/day
b Chloroquine 1x/day for days 1,2 and 3 only

TABLE 30

DETAILED ACTIVITY OF RE-TREATMENT WITH WR 256287AA (BL 28636) /WR 256287AB (BL 51153) PLUS CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH /RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
11969r	50.0a 20.0b	14	65	45	25	0.4	0.3	<0.01	<0.01	<0.01	0.8	0.6
12204r	50.0a 20.0b	65	58	75	12	1	0.9	0.6	<0.01	0	<0.01	<0.01

a. WR 256287 1x/day
b. Chloroquine 1x/day

TABLE 31

SUMMARY OF THE ACTIVITY OF WR 256287AA (BL 28636)/WR 256287AB (BL 51153) PLUS CHLOROQUINE, (WR 00154BM; AR 20613), AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
11969	20.0a 20.0b		+		n.a.	n.a.	Re-Rx, higher dose
12204	20.0a 20.0b		+		n.a.	n.a.	Re-Rx, higher dose
11969r	50.0aa 20.0bb			+	14	55	
12204r	50.0aa 20.0bb			+	13	48	
12178	20.0b	+			n.a.	n.a.	Died day 4 Post Rx, malaria

a. WR 256287 - 3x/ day for 7 days
b. Chloroquine 1x/day for Days 1-2-3
aa. WR 256287 1x/day for 3 days
bb. Chloroquine 1x/day for 3 days

TABLE 32

ACTIVITY OF WR 256287AA (BN:BL 28636) AND WR 256287AB (BN:BL 51153), ALONE, AND IN COMBINATION WITH CHLOROQUINE (WR 001544BM; BN:AR 20613) AGAINST TWO STRAINS OF PLASMODIUM FALCIPARUM

MALARIA STRAIN	DOSE mg/kg TOTAL	DOSE mg/kg DAILY	PRIMARY TREATMENTS CLEARED	PRIMARY TREATMENTS CURED	REPEAT TREATMENTS CLEARED	REPEAT TREATMENTS CURED	TOTAL TREATMENTS CLEARED	TOTAL TREATMENTS CURED
VIETNAM SMITH	3.0	1.0a	0/1	0/1			0/1	0/1
	75.0	25.0a	0/1	0/1			0/1	0/1
	3.0 60.0	1.0a 20.0b	0/1	0/1			0/1	0/1
	15.0 60.0	5.0a 20.0b			2/2	1/2	2/2	1/2
	75.0 60.0	25.0a 20.0b	0/1	0/1			0/1	0/1
	150.0 60.0	50.0a 20.0b			2/2	1/2	2/2	1/2
	60.0	20.0b	0/1	0/1	1/1	0/1	1/2	0/2
SMITH/RE	420.0 60.0	60.0aa 20.0b	0/2	0/2			0/2	0/2
	150.0 60.0	50.0a 20.0b			2/2	0/2	2/2	0/2
	60.0	20.0b	0/1	0/1			0/1	0/1

a. WR 256287 1x/day
aa. WR 256287 3x/day for 7 days
b. Chloroquine 1x/day for 3 days

G. WR 256410AA; BN:BL 30887

This drug is a chlorpromazine analog prepared by Smith Kline and French Laboratories and was evaluated against Vietnam Smith infections for its potential to reverse chloroquine resistance *in vivo* (Tables 33 - 35). When WR 256410 was administered alone in a 3-day dosage of 5.0 mg per kg, there was no response of the parasitemia; retreatment with 10.0 mg per kg of the drug was initiated,and the monkey died of malaria on day 3 of treatment. Primary treatment with the drug alone at 10.0 mg per kg (x 3 days) had no effect upon the parasitemia, while retreatment with 20.0 mg per kg (x 3) cleared the parasitemia, but a recrudescence occurred.

Combined treatment for three days with WR 256410 (5.0 mg per kg) plus chloroquine (20.0 mg per kg) had questionable suppressive activity on the parasitemia. Retreatment with the experimental drug (10.0 mg per kg x 3 days) and chloroquine (20.0 mg/kg x 3 days) cleared the parasitemia, but the infection was not cured. A 3-day primary treatment of WR 256410 (10.0 mg per kg) plus 20.0 mg per kg of chloroquine suppressed parasitemia, and retreatment with 20.0 mg per kg each of the experimental drug plus chloroquine cleared the parasitemia, but with a subsequent recrudescence.

TABLE 33

DETAILED ACTIVITY OF WR 256410AA (BL 30887), ALONE, AND IN COMBINATION WITH CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
10941	5.0a	0.4	12	11	91	168	439	518	Re-Rx, higher dose			
10941r	10.0b	158	432	419	Died- malaria							
10813	10.0a	0.6	11	40	82	159	413	641	Re-Rx, higher dose			
10813r	20.0b	641	567	542	160	187	161	57	30	43	498	17
12268	5.0a	0.2	20	36	99	149	225	419	Re-Rx, higher dose			
	20.0b											
12268r	10.0a	419	142	271	192	91	11	5	1	0.3	0.6	0.1
	20.0b											
12286	10.0a	0.9	57	26	170	95	204	160	Re-Rx, higher dose			
	20.0b											
12286r	20.0a	160	222	259	353	234	348	35	18	28	43	22
	20.0b											

a WR 256410
b Chloroquine

TABLE 34

SUMMARY OF THE ACTIVITY OF WR 256410AA (BL 30887), ALONE, AND IN COMBINATION WITH CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
10941	5.0a	+			n.a.	n.a.	Re-Rx, higher dose
10941r	10.0a	+			n.a.	n.a.	Died Day 3 of Rx
10813	10.0a	+			n.a.	n.a.	Re-Rx, higher dose
10813r	20.0a			+	15	26	
12268	5.0a 20.0b		±		n.a.	n.a.	Re-Rx, higher dose
12268r	10.0a 20.0b			+	10	45	
12286	10.0a 20.0b		+		n.a.	n.a.	Re-Rx, higher dose
12286r	20.0a 20.0b			+	14	21	

a= WR 256410
b= Chloroquine

TABLE 35

ACTIVITY OF WR 256410AA (BN:BL 30887), ALONE, AND IN COMBINATION WITH CHLOROQUINE (WR 001544BM; BN:AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

MALARIA	DOSE mg/kg		PRIMARY TREATMENTS		REPEAT TREATMENTS		TOTAL TREATMENTS	
STRAIN	TOTAL	DAILY	CLEARED	CURED	CLEARED	CURED	CLEARED	CURED
'IETNAM SMITH	15.0	5.0a	0/1	0/1			0/1	0/1
	30.0	10.0a	0/1	0/1	0/1	0/1	0/2	0/2
	60.0	20.0a			1/1	0/1	1/1	0/1
	15.0 60.0	5.0a 20.0b	0/1	0/1			0/1	0/1
	30.0 60.0	10.0a 20.0b	0/1	0/1	1/1	0/1	1/2	0/2
	60.0 60.0	20.0a 20.0b			1/1	0/1	1/1	0/1

a WR 256410
b Chloroquine

H. WR 255693AC; BN:BL 48567 - Diltiazem

Results of the trial to reverse chloroquine resistance in infections with the Vietnam Smith strain are presented in Tables 36 and 37. Diltiazem was administered in a 3-day dosage at 5.0 mg per kg three times a day. Chloroquine was administered on day 1 at 40.0 mg per kg, and on days 2 and 3 at 20.0 mg per kg. Primary treatment with diltiazem plus chloroquine suppressed the parasitemia in each of two Aotus, and retreatment cleared the parasitemia, but the infections were not cured. Parasitemia was suppressed during primary treatment in the monkeys that received chloroquine only; retreatment cleared the parasitemias, but did not cure the infections.

The data in Tables 38 and 39 indicate the results of diltiazem plus chloroquine evaluated against infections of the Vietnam Smith/RE strain. Diltiazem was administered three times per day for three days and chloroquine administered once daily at 20.0 mg per kg for three days. Primary treatment with diltiazem (5.0 mg per kg) plus chloroquine suppressed parasitemia in each of two Aotus. Retreatment with diltiazem (10.0 mg per kg) plus chloroquine had no activity against parasitemia in one monkey, that died of malaria on day 1 post treatment; the parasitemia was cleared in the second animal, that died of an intercurrent infection on day 13 post treatment.

Primary treatment with chloroquine only had no effect upon parasitemia in one Aotus that died of malaria on day 4 post treatment. The parasitemia was suppressed in the second animal that received chloroquine only and the infection was cured following retreatment.

TABLE 36

DETAILED ACTIVITY OF DILTIAZEM IN COMBINATION WITH CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12304	5.0a 40/20b	5	59	14	14	5	80	31	231	Re-Rx, same dose		
12704r	5.0a 40/20b	231	296	332	302	167	197	105	82	6	0.5	0.1
12316	5.0a 40/20b	6	91	51	2	0.2	0.01	0.01	0.01	Re-Rx, same dose		
12316r	5.0 40/20b	<0.01	1	0.05	<0.01	<0.01	<0.01	<0.01	0.3	0.1	<0.01	<0.01
12328	40/20b	10	99	82	15	1	12	30	161	Re-Rx, same dose		
12328r	40/20b	161	221	142	87	11	29	2	3	3	3	26
12329	40/20b	10	112	73	0.6	0.01	0.01	0.01	0.6	Re-Rx, same dose		
12329r	40/20b	0.6	0.8	2	0.1	0.6	2	0.4	0.6	0.2	0.3	<0.01

a. Diltiazem - 3x/day
b. Chloroquine 40.0mg/kg Day 1, 20.0 mg/kg days 2 and 3.

TABLE 37

SUMMARY OF THE ACTIVITY OF DILTIAZEM IN COMBINATION WITH CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
12304	5.0a 40.0/20.0b		+		n.a.	n.a.	Re-Rx, same dose
12304r	5.0a 40.0/20.0b			+	14	30	
12316	5.0a 40.0/20.0b		+		n.a.	n.a.	Re-Rx, same dose
12316r	5.0a 40.0/20.0b			+	14	109	
12328	40.0/20.0b		+		n.a.	n.a.	Re-Rx, same dose
12328r	40.0/20.0b			+	15	34	
12329	40.0/20.0b		+		n.a.	n.a.	Re-Rx, same dose
12329r	40.0/20.0b			+	14	27	

a. Diltiazem - 3x/day
b. Chloroquine - 40w0 mg/kg Day 1, 20.0 mg/kg Days 2 and 3.

TABLE 38

DETAILED ACTIVITY OF DILTIAZEM (WR 255693AC; BL 48567) PLUS CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
11949	5.0a 20.0b	9	33	50	18	28	63	65	172	111	Re-Rx, higher dose	
11949r	10.0a 20.0b	111	111	228	80	111	75	86	117	42	1	0.7
11967	5.0a 20.0b	16	23	52	40	60	142	91	284	137	Re-Rx, higher dose	
11967r	10.0a 20.0b	137	838	326	468	227	Died-malaria					
11921	20.0b	16	71	100	221	418	961	887		Died-malaria		
11932	20.0b	14	49	74	55	152	136	99	19	130	Re-Rx, same dose	
11932r	20.0b	130	136	111	79	86	49	111	4	0.9	< 0.01	0

a Diltiazem - 3x/day
b Chloroquine - 1x/day

TABLE 39

SUMMARY OF THE ACTIVITY OF DILTIAZEM (WR 255693AC; BL 48567) PLUS CHLOROQUINE (WR 1544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
11949	5.0a 20.0b		+		n.a.	n.a.	Re-Rx, higher dose
11949r	10.0a 20.0b			+	12		Died Day 13-Post-Rx*
11967	5.0 20.0b		+		n.a.	n.a.	Re-Rx, higher dose
11967r	10.0 20.0b	+					Died Day 1 Post-Rx, malaria
11921	20.0b	+			n.a.	n.a.	Died Day 4 Post Rx, malaria
11932	20.0b		+		n.a.	n.a.	Re-Rx, same dose
11932r	20.0b			+	10	n.a.	Cured

a. Diltiazem, 3 x/day
b. Chloroquine, daily dose
* Intercurrent infection

TABLE 40

ACTIVITY OF DILTIAZEM (WR 255693AC; BN:BL 48567) PLUS CHLOROQUINE (WR 001544AM; BN:AR 20613) AGAINST INFECTIONS OF TWO STRAINS OF PLASMODIUM FALCIPARUM

MALARIA STRAIN	DOSE mg/kg TOTAL	DOSE mg/kg DAILY	PRIMARY TREATMENTS CLEARED	PRIMARY TREATMENTS CURED	REPEAT TREATMENTS CLEARED	REPEAT TREATMENTS CURED	TOTAL TREATMENTS CLEARED	TOTAL TREATMENTS CURED
VIETNAM SMITH	45.0 80.0	15.0a 40/20/20b	0/2	0/2	2/2	0/2	2/4	0/4
	80.0	40/20/20b	0/2	0/2	2/2	0/2	2/4	0/4
SMITH/RE	45.0 60.0	15.0a 20.0bb	0/2	0/2			0/2	0/2
	90.0 60.0	30.0aa 20.0bb			1/2	0/2	1/2	0/2
	60.0	20.0bb	0/2	0/2	1/1	1/1	1/3	1/3

a Diltiazem 5.0 mg/kg 3x/day
aa Diltiazem 10.0 mg/kg 3x/day
b Chloroquine 40.0 mg/kg Day 1, 20.0 mg/kg Days 2 and 3
bb Chloroquine - daily dose

I. WR 256975AA; BN:BL 39755 - Bepridil

This calcium channel blocker was evaluated against infections of the Vietnam Smith strain of P. falciparum (Tables 41-43). Bepridil was administered 3 times per day for three days and chloroquine administered on day 1 at 40.0 mg per kg, and on days 2 and 3 at 20.0 mg per kg.

Bepridil only (8.0 mg per kg) had no effect upon the parasitemia in Aotus 11327 and the animal died on day 5 post treatment of malaria. Some suppression of parasitemia was noted in Aotus 12047 that received bepridil (8.0 mg per kg) and retreatment with bepridil (16.0 mg per kg) plus chloroquine cleared the parasitemia, but did not cure the infection.

Primary treatment with bepridil (8.0 mg per kg) plus chloroquine suppressed the parasitemia in monkeys 12324 and 12325. Retreatment with bepridil (16.0 mg per kg) plus chloroquine cleared the parasitemia in each Aotus, but did not cure the infection.

Parasitemia was suppressed by chloroquine treatment in Aotus 12328 and 12329 and retreatment cleared the parasitemias, but without infection cure.

TABLE 41

DETAILED ACTIVITY OF BEPRIDIL (WR 256975AA; BL 39755), ALONE, AND IN COMBINATION WITH CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
11327	8.0a	5	73	15	284	166	259	542	284	Died - malaria		
12047	8.0a	3	35	19	141	81	172	286	142	Re-Rx, higher dose plus CQ		
12047	16.0a 40/20b	142	283	53	I	0.08	2	6	1	0.3	<0.01	<0.01
12324	8.0a 40/20b	5	56	57	3	0.1	<0.01	<0.01	0.3	Re-Rx, higher dose		
12324r	16.0a 40/20b	0.3	4	0.5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.3	1
12325	8.0a 40/20b	6	66	73	1	0.04	<0.01	<0.01	0.1	Re-Rx, higher dose		
12325r	16.0a 40/20b	0.1	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
12328	40/20b	10	99	82	15	1	12	30	161	Re-Rx, same dose		
12328r	40/20b	161	221	142	87	11	29	2	3	3	3	26
12329	40/20b	10	112	73	0.6	0.01	0.01	0.01	0.6	Re-Rx, same dose		
23329r	40/20b	0.6	0.8	2	0.1	0.6	2	0.4	0.6	0.2	0.3	<0.01

a WR 256975 - 3x/day
b Chloroquine 40.0 mg/kg day 1, 20.0 mg/kg - days 2 and 3
CQ= Chloroquine

TABLE 42

SUMMARY OF THE ACTIVITY OF BEPRIDIL (WR 256975AA: BL 39755), ALONE, AND in COMBINATION WITH CHLOROQUINE (WR 001544BM; AR 20613) AGAINST INFECTIONS OF THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
11327	8.0a	+			n.a.	n.a.	Died Day 5 Post-Rx, malaria
12047	8.0a		±		n.a.	n.a.	Re-Rx, higher dose plus chloroquine
12347r	16.0a 40/20b			+	23	40	
12324	8.0a 40/20b		+		n.a.	n.a.	Re-Rx, higher dose
12324r	16.0a 40/20b			+	28	47	
12325	8.0a 40/20b		+		n.a.	n.a.	Re-Rx, higher dose
12325r	16.0a 40/20b			+	14	15	
12328	40/20b		+		n.a.	n.a.	Re-Rx, same dose
12328r	40/20b			+	15	34	
12329	40/20b		+		n.a.	n.a.	Re-Rx, same dose
12329r	40/20b			+	14	27	

a. Bepridil - 3 x/day
b. Chloroquine - 40.0 mg/kg Day 1, 20.0 mg/kg Days 2 and 3.

TABLE 43

ACTIVITY OF BEPRIDIL (WR 256975AA; BN:BL 39755), ALONE AND IN COMBINATION WITH CHLOROQUINE (WR 001544BM; BN:AR 20613) AGAINST PLASMODIUM FALCIPARUM INFECTIONS

MALARIA STRAIN	DOSE mg/kg TOTAL	DOSE mg/kg DAILY	PRIMARY TREATMENTS CLEARED	PRIMARY TREATMENTS CURED	REPEAT TREATMENTS CLEARED	REPEAT TREATMENTS CURED	TOTAL TREATMENTS CLEARED	TOTAL TREATMENTS CURED
VIETNAM SMITH	72.0	24.0a	0/2	0/2			0/2	0/1
	72.0 80.0	24.0a 40/20/20b	0/2	0/2			0/2	0/2
	144.0 80.0	48.0aa 40/20/20b			2/3	0/3	2/3	0/3
	80.0	40/20/20b	0/2	0/2	2/2	0/2	2/4	0/4

a Bepridil 8.0 mg/kg 3x/day
aa Bepridil 16.0 mg/kg 3x/day
b Chloroquine 40.0 mg/kg Day 1, 20.0 mg/kg Days 2 and 3

J. Conclusions

The aim of these experiments was to determine if chloroquine resistance in infections with a chloroquine resistant strain of P. falciparum could be reversed in vivo by the co-administration of a calcium channel blocker and chloroquine. Ideally, reversal of resistance would be indicated by parasite clearance after one course of combined treatment during the ascending phase of the primary patent period, and no recrudescence, thus signifying infection cure. In a total of 26 combined treatments, during the primary patent period, suppression of parasitemia occurred in 17 monkeys. Verapamil plus chloroquine cleared the parasitemia in each of two Aotus and the infection was cured in one monkey. The second monkey died of an intercurrent infection on day 29 post treatment.

It should be noted, however, that the time for parasite clearance between the day treatment was initiated and the last parasite-positive blood film was 21 and 19 days, respectively. Based upon previous experiments, in which a drug cleared a primary Vietnam Smith parasitemia and cured the infection, the time for parasite clearance in 60 trials was 7.3 (± 1.1) days. It is possible that the protracted parasite clearance time for verapamil plus chloroquine was the result of both acquired immunity and chemotherapy. Moreover, the primary patent period in 70 untreated Panamanian Aotus infected with the Vietnam Smith strain and that exhibited self cure following the primary attack was 25.0 (± 4.4) days (6). The primary patent periods in the two Aotus administered verapamil plus chloroquine with parasite clearance were 23 and 21 days, respectively.

In a total of 28 repeat treatments, with a channel blocker and chloroquine, infections in 6 (21%) Aotus were cured, as indicated by the absence of recrudescence. These retreatments were inititated during the primary patent period, following lack of parasite response to the first drug dosage. Again, it is difficult to separate the drug activity from acquired immunity. The primary patent period in the six Aotus was 23.2 (± 0.7) days, essentially identical to that in untreated Aotus, infected with the Smith strain, and with self-cure after the primary attack.

The failure to achieve the desired goal in these experiments may be attributable to at least two factors:

1. Inability to obtain, in the monkey, the high levels of drugs as reported in the in vitro studies and still sustain host viability.

2. The metabolism of these drugs in Aotus is unknown and conversion to inactive constituents may occur.

Additional trials with other calcium channel blockers, or similar acting drugs, are anticipated during the second year of this contract.

LITERATURE CITED

1. Moore, D. V. and Lanier, J. E. 1961. Observations on two Plasmodium falciparum infections with an abnormal response to chloroquine. Am. J. Trop. Med Hyg. 10: 5-9.

2. Young, M. D. and Moore, D.V. 1961. Chloroquine resistance in Plasmodium falciparum. Am. J. Trop. Med Hyg. 10: 317-320.

3. Martin, S K., Oduola, A. M. J.and Milhous, W. K. 1987. Reversal of chloroquine resistance in Plasmodium falciparum by verapamil.Science. 235: 899-901.

4. Krogstad, D. J., Gluzman, I. Y., Kyle, D. E., Oduola, A.M.J., Martin, S. K., Milhous, W. K. and Schlesinger, P. H. 1987. Efflux of chloroquine from Plasmodium falciparum: mechanism of chloroquine resistance. Science . 238: 1283-1285.

5. Schmidt, L. H. 1978. Plasmodium falciparum and Plasmodium vivax infections in the owl monkey (Aotus trivirgatus). II. Responses to chloroquine, quinine, and pyrimethamine. Am. J. Trop. Med. Hyg. 27: 703-717.

6. Rossan, R. N., Harper, J. S. III, Davidson, D. E. Jr, Escajadillo, A., and Christensen, H. A. 1985. Comparison of Plasmodium falciparum infections in Panamanian and Colombian owl monkeys. Am. J. Trop. Med Hyg. 34: 1037-1047.

DISTRIBUTION LIST

5 copies	Director Walter Reed Army Institute of Research Walter Reed Army Medical Center ATTN: SGRD-UWZ-C Washington, DC 20307-5100
1 copy	Commander US Army Medical Research and Development Command ATTN: SGRD-RMS Fort Detrick, Frederick, Maryland 21701-5012
2 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDAC Cameron Station Alexandria, VA 22304-6145
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20814-4799
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234-6100